

TECHNICAL HANDBOOK

XNAPS Micro DNA Flexbond Kit

Catalog Number P1038

Total DNA purification from
small amounts of biological samples

Version 2009

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All technical literature and related information are available on the website: www.renogenbio.com

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Kit Contents

Catalog Number	P1038
Kit Size	100 preps
Lysis Buffer LB	30 ml
Denaturation buffer DB	15 ml
DNA binding Resin	30 ml
Column Wash Buffer WB	24 ml
Nuclease-free water	20 ml
Proteinase K solution	1 ml
Filter columns	100
Collection tubes	100
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Storage Conditions

Upon received, Proteinase K solution should be stored at -20°C. Store the DNA Binding Resin at 2-8°C. All other buffers and components can be stored at room temperature for one year without any reduction of performance.

Quality Control

In addition to routine monitoring and detection of the kit components, the performance of XNAPS micro DNA

flexbond kits are tested on a lot-to-lot basis by purification of total DNA from 100 µl of overnight –cultured *E. coli*. The yield and purity of purified DNA is checked by agarose gel electrophoresis and spectrophotometrical analysis.

Safety Precautions

Although no toxic reagents are contained in the serial XNAPS kits, all chemicals should be considered as potentially hazardous. All due care and attention should be exercised in handling the materials and reagents in the kit. We recommend users always wear laboratory coat, safety glasses, and gloves. In the case of contact with skin or eyes, wash immediately with a large amount of water.

Technical Assistance

We encourage our customers to contact us by any means of telephone, fax, mail/email. Our experienced staff are always ready to assist you about any questions and problems derived from our products. Also, you can find most of the information and data of Renogen products from our website.

Contacting information:

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Introduction

XNAPS micro DNA flexbond kit provides an efficient and fast method for isolating DNA from very small amounts of biological samples, such as 1-100 µl of whole blood, 100 µl of serum, 3-10mg of animal tissue, 100µl of urine, saliva spot, sperm spot, etc.

The system combines a modified enzymatic lysis procedure with an innovative chromatographic absorbent resin which preferentially binds DNA with high capacity. After thorough lysis of samples by chemical/enzymatic lysis buffer and denaturation of contaminants, the released free- form DNA binds specifically to DNA binding resin in aqueous solutions. Impurities are then washed away from the resin. The binding DNA is eluted in micro volume of water.

The novel procedure provided by the kit overcomes main disadvantages, such as time-consuming methods, toxic components, low yield, as compared to other commercial kits. The purified DNA is immediately ready to use in various downstream applications, such as PCR, restriction enzyme digestion, sequencing.

Key Features

- **Speed:** All the processing steps can be completed within 30 minutes.
- **Micro elution volume:** As little as 25µl can be used to elute target DNA.
- **High yield and purity:** The OD₂₆₀/OD₂₈₀ of purified DNA is >1.8.
- **Safety :** No phenol extraction, no ethanol precipitation, no toxic chaotropic salts.
- The purified DNA is ready for downstream manipulations.
- **No spin column is needed.**

Protocols

The principle of XNAPS Micro DNA flexbond kit is totally different from the methods of other suppliers. Do not exchange or replace the components of XNAPS Micro DNA flexbond kit with components from any other suppliers.

Materials to be supplied by the user

Microcentrifuge capable of 14000 x g

55°C water bath or heating block

Ethanol(>95%)

Sterile 1.5ml centrifuge tubes

Ice

(optional) RNase A solution (10µg/ml)

Prior to starting:

1. Add 96 ml of ethanol(>95%) to each wash buffer WB.
2. Preheat a water bath or heating block to 60°C.
3. Examine the buffers in the kit. Buffers may form precipitation in low storage temperature. If there is a precipitate, warm at up to 60°C until the solution becomes clear.

I. Lysis of starting samples

I.I. Liquid samples

1. Transfer up to 150 µl of starting sample into a sterile 1.5 ml centrifuge tube.

Liquid materials include clinical samples (e.g. whole blood, milk, plasma, serum, saliva, urine, sputum, cerebrospinal fluid), environmental samples (e.g. water), cultured samples (e.g. supernatant of cell culture), or dirty DNA solution.

2. Add 150 µl of lysis buffer LB to the tube. Close the tube tightly and vortex briefly to mix the solution thoroughly. *If LB is precipitated or cloudy before use, heat the solution up to 60°C until it becomes clear.*
3. Add 10 µl of Proteinase K solution. Mix thoroughly by vortexing.
4. Incubate the tube at 55°C for 15 minutes.

I.II. Solid samples

1. Transfer the solid sample into a sterile 1.5 ml centrifuge tube.

Solid materials include such as soil, dried blood spot, saliva spot, sperm spot, swab

2. Add 150 µl of buffer TE. Close the tube tightly and Vortex vigorously for 10 seconds.

3. Add 150 µl of lysis buffer LB to the tube. Close the tube tightly and vortex briefly to mix the solution thoroughly. *If LB is precipitated or cloudy before use, heat the solution up to 60°C until it becomes clear.*
4. Add 10 µl of Proteinase K solution. Mix thoroughly by vortexing.
5. Incubate the tube at 60°C for 30 minutes.

I.III. Animal tissues

1. Transfer up to 10 mg of animal tissue, either fresh or frozen, into a 1.5 ml centrifuge tube.
2. Add 300 µl of buffer LB to the tube.
3. Add 10 µl of Proteinase K solution. Mix thoroughly by vortexing.
4. Incubate the tube at 55°C for overnight, or until the tissue is completely lysed.

Most samples can be lysed completely in 2-3 hours, however, lysis with longer time (but less than 16 hours) can release more DNA .

II. Preparation of clear lysate

1. After lysis, add 150 µl of buffer DB to the tube. Close the tube and mix thoroughly by vortexing for 5 seconds.

The solution should become cloudy with white precipitates.

2. Centrifuge the lysate at maximum speed at room temperature for 10 minutes.

Clear supernatant should be formed.

III. Isolation of Total DNA

1. While the sample is in centrifugation, insert a filter column into a collection tube.
2. Transfer 300 µl of DNA binding resin into the filter column.

Note: *Resuspend the resin thoroughly by vortexing or shaking before transferring.*

3. After centrifugation, transfer the supernatant into the filter column by decanting or pipetting. Mix the suspension thoroughly by pipetting 4-5 times. Incubate at room temperature for 2 minutes .
4. Centrifuge at maximum speed for 1 minute . Discard the flowthrough.
5. Add 600 µl of wash buffer WB into the column. Centrifuge at maximum speed for 1 minute . Discard the flowthrough.

NOTE: *Ensure that ethanol has been added to buffer WB.*

6. Repeat the above washing once. Discard the flowthrough from the collection tube. Reinsert the column into the collection tube.
7. Centrifuge at maximum speed for 2 minutes to remove residual washing buffer .
8. Transfer the column to a new sterile 1.5 ml microcentrifuge tube.
9. Add 100 of nuclease-free water into the center of the spin column. Stand at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute.

Tips for efficient elution:

Use prewarmed(60°C) water can increase the yield. Or elute DNA two times with 50 µl of water each time.

To get more concentrated DNA, add 50 µl of prewarmed water and incubate the column at room temperature for 2 minutes before centrifugation.

10. The purified DNA is ready for downstream application. Or, store the purified DNA at -20°C for later use. For longer storage, addition of 1/10 volume of 10 x TE is recommended.

Yield and purity Examination

Both spectrophotometrical analysis and agarose gel electrophoresis are recommended for the yield and purity determination of the purified DNA. To determine the concentration of DNA by spectrophotometer, the following formula should be used :

$$[\text{DNA}] (\mu\text{g/ml}) = A_{260} \times 50 \times D,$$

where D is the dilution factor.

The yield of DNA can be calculated by multiplying the concentration by the volume of DNA solution.

The DNA purified by XNAPS Micro DNA flexbond kit should be of high purity with the ratio of $OD_{260}/OD_{280} > 1.8$. The capacity of DNA binding for each prep is 25 μg .

Supplementary Information

Composition of buffer TE (pH8.0)

10mM Tris-Cl(pH8.0)

1mM EDTA(pH8.0)

Enhancement of PCR performance

bovine serum albumin (BSA) can enhance the downstream PCR performance effectively. Addition of BSA to PCR mixtures to a final concentration of 0.1-1.0 $\mu\text{g}/\mu\text{l}$ may allow

positive amplifications for some difficult templates purified from highly contaminated starting materials .

Removal of co-purified RNA

For DNA purification from starting samples rich of RNA, such as from animal tissues, or if absolute control of RNA contamination is necessary, the following two methods can be applied to remove contaminated RNA:

1. After complete lysis (see protocol), add 10 μl of RNase A solution (10mg/ml, DNase-free, supplied by user) and incubate at room temperature for 15 minutes. Then proceed to step 1 of II. Preparation of clear lysate
2. Add 1 μl of above RNase A solution to the final eluted DNA solution.

Troubleshooting

Problem	Possible Causes	Comments
Low yield or no DNA in elute	Too small amount of starting material	Use more amount of starting material
	Insufficient lysis	Use fresh prepared Proteinase K solution(20mg/ml)
	Ethanol omitted from Wash Buffer	Add ethanol as described
	Poor elution	Add 30 µl of water and incubate at 75°C for 3 minutes
	sample is too old	Use fresh sample
RNA contamination	Insufficient RNase A digestion	See "removal of RNA contamination" in Supplementary Information
DNA does not perform well in downstream applications	Low yield or no DNA in elute	See above " Low yield or no DNA in elute"
	Inhibitor in elute	Ensure complete removal of WB at each washing step
		Re-cleanup of elute

Product Use Limitations

XNAPS Micro DNA flexbond kit is developed and sold for research purpose only. It is not to be used for human diagnostic or drug purposes or to be administered to humans and animals. The user is responsible to validate the performance of the system for any specific applications.

Product warranty

Renogen guarantees the performance of all products for applications as described in the technical handbook. If any product fails to perform as described due to any reason, other than misuse, we will replace it free of charge or refund the purchase price.

We reserve the rights to change, alter, or modify our products to enhance its performance and design. If you have any concerns about Renogen products and services, please contact us by telephone, fax, mail, or email.

Ordering Information

Customers in USA and Canada

To place an order, please use any of the following ways:

Phone: 1-866-712-4412(Toll free)
Mon.-Fri 8:00am-5:00pm (EST)
Fax: 1-651-204-9348
Mail: #310, 2386 East Mall
Vancouver, BC, V6T 1Z3
Canada

Customers out of USA and Canada

Please contact our authorized international distributors and local representatives. In areas without our distributors and representatives, following options are available:

Phone: 1-651-204-0326
Mon.-Fri 8:00am-5:00pm (EST)
FAX: 1-651-204-9348
Mail: #310, 2386 East Mall
Vancouver, BC, V6T 1Z3
Canada

Online Ordering

Ordering for all of our products from any places is available, 24 hours/day, 7 days/week. Online ordering is fast, and convenient. Please log on www.renogenbio.com for detailed information.